AD 685891

TRANSLATION NO. 2400

DATE: 26 Feb 1969

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PROBLEMS OF Virology 11(1): 1966, p.21-25 #2400

THE INFLUENCE OF HISTONE ON THE INTRACELLULAR DEVELOPMENT OF THE VACCINIA VIRUS

I. G. Balandin, I. A. Kozlova, L. A. Mel'nikova, L. V. Masharina, O. P. Peterson and V. M. Zhdanov, Institute of Biologic and Medical Chemistry of USSR Acad, Med, Sci, and "Ivanovskii" Institute of Virusology of USSR Acad, Med. Sci., Moscow.

That rapid and considerable depression of synthesis of intracellular proteins and nucleic acids occurs shortly after infecting cells with various RNA-containing viruses has been established in a fairly large number of experiments (6,11,12,15). The reduced synthesis of cellular RNA is apparently due to the appearance of an inhibitor of protein nature in the infected cells. It has been suggested that this protein is a histone (5), which raises the question of the influence of histone on the development of the virus itself. As regards RNA-containing viruses, experiments with Sendai virus have shown that pretreatment of cells with histone accelerates the onset of the phase of rapid synthesis of viral RNA- (1). We did not, however, find any information in the available literature regarding the influence of histone on the replication of DNA-containing viruses.

Our aim was to study the influence of histone on the development of DNA-containing viruses in tissue culture.

MATERIALS AND METHODS

The vaccinia virus (dermovaccine strain) was used for infecting 48-hr tube cultures of chick fibroblasts (800,000 cells in each tube) in nutrient medium 199 containing 5% bovine serum. Cells were infected with various amounts of the virus (from 0.1 to 12 ID per cell). Absorption of virus continued for an hour, and then the cells were repeatedly rinsed free of virus with large amounts of physiologic saline.

The histone used in the experiments was extracted with 0.25 N H_2SO_4 from the thymus gland of a calf (14), and the extract was dialyzed and lyophilized. The product dissolved readily in water and in the culture medium. Histone (100 ug/ml) was added to the medium at various times before and after infecting the cells with the virus as well as simultaneously with the virus.

When histone was introduced before infection, medium containing histone was decanted before starting adsorption, stored at low temperature throughout the entire period of adsorption and again added to the tubes after rinsing. In the case of simultaneous introduction of histone and virus the adsorption of the latter occurred in the medium containing histone, after which the cells were rinsed clear of unabsorbed virus and then covered over with medium containing the same amount of histone. In experiments where histone was added after infection of the cells it was introduced in a small volume of medium for a final concentration of histone in the medium of 100 ug/ml.

Incubation was at 37° ; for 24 hr after infection the culture medium was then removed and the cells were washed with physiologic saline and suspended in MacIlvain's solution of pH 7.2. The quantity of the latter was equal to the amount of medium in which incubation was performed. The cells were disrupted by alternate freezing and thawing, and the virus was titrated on chorioallantoic membranes of 11-day chick embryos.

In control experiments the same procedures were carried out, but without the use of histone.

Results

Histone (100 ug/ml)

Histone (100 ug/ml) introduced at t he start of incubation, prevented multiplication of the vaccinia virus. Study of the influence of the duration of incubation with histone on the subsequent synthesis of the virus showed that even a transient contact (5-15 min) with this protein decreased the subsequent multiplication of the virus. Longer contact with histone resulted in a considerable and sustained depression of vaccinia virus synthesis (Table 1).

The introduction of histone at various time intervals after infection of a tissue culture with the virus stopped further synthesis of the virus, and the amount of the latter, determined by means of titration, evidently corresponded to the amount of the virus which could be synthesized by the moment of introduction of histone (Table 2).

Experiments with introduction of histone before infection of cells with the virus enabled one to ascertain the duration of the effective action of histone. It was found that histone (100 ug/ml) prevented synthesis of the virus if the cells were treated with it 1-2 hr before infection. The interval between infection and introduction of histone was prolonged the inhibiting effect of the latter progressively decreased; histone added to the cells at a rate of 100 ug/ml 12 hr after infection had no inhibitory effect at all on the development of the virus (Table 3).

Discussion of the results

The action of histone on DNA is due to its ability to block the negative charges of the molecules of DNA, and this leads to a marked inhibition of metabolic activity of the latter (2, 3, 7, 13). The complex

of DNA + histone loses not only the ability to secure replication of DNA (9) but also the matrix function of DNA during the synthesis of RNA, including information RNA (4,10,13). One can not exclude the possibility of a direct influence of histone on the activity of various intracellular enzymes and of enzymes responsible for the synthesis of the viral components, due to the capacity of histones to form complexes with proteins (8,16,17).

The inhibitory action of histone on the development of DNA-containing virus, of which the vaccinia virus is an example, can apparently be explained by the blocking of DNA of this virus. Nucleic acid present inside the virus is in all probability insensitive to the action of histone or rather is inaccessible to the latter, as is indicated by the results of adding histone at various time intervals after infecting the cells with the virus.

The least quantity of virus was found in cases where histone was added at ½ - 4 hr after infection. Apparently this "residual" infectivity was due to virions which did not "divide" in the cells, so that their nucleic acid was not influenced by histone. The fact that with simultaneous introduction of histone and of virus infectivity was higher than when histone was added 1-4 hr after infection might be because in the former case histone prevented stripping of the virus, either preventing formation of the enzyme which strips the virus (matrix-blocking) or forming with such enzyme an inactive complex (direction action).

Histone added after 4 hr evidently blocked the free DNA without affecting the virions already formed so that it was possible to follow the trend of the development of the virus within the cell. In other words, the curve contructed from the results of titration of the virus in experiments with addition of histone at various time intervals after infection reflects the trend of multiplication of the virus and of accumulation of

mature viruses. (Fig, I.).

Histone occurring in the cells in "free" (not bound with DNA) state gradually loses its blocking activity (complete loss of this activity was noted in experiments after 12 hr), as evidenced by data obtained in study of the synthesis of the virus in cells pretreated with histone. It is possible that histone in these cases is gradually destroyed by the proteolytic enzymes of the cells. At the same time, the DNA-histone complex already formed is preserved for a much longer time. For example, with simultaneous introduction of histone and virus (or addition of histone 1-2 hr after infection) the resultant DNA-histone complex was maintained for at least 24 hr. This was evidenced by the fact that titration carried out after this time interval did not reveal formation of mature virus, as would be expected in the case of the instability or reversibility of the DNA-histone complex.

SUMMARY

Histone, isolated from the calf thymus gland, possesses the ability to block the synthesis of DNA-containing viruses, apparently by forming a histone-virus DNA complex , thereby excluding the virus-DNA from the process of reproduction of the virus.

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Received May 8, 1965

Table 1

Influence of the duration of contact of cells with histone on subsequent development of vaccinia virus

Time of contact with histone	Titer of virus				
	undiluted				
Control					
5 min.					
15 min.					
30 min.					
1 hr.					
1½ hr.					
3 hr.					

Notes: The infecting dose of vaccinia virus was 12 ID per cell. Histone was added to the medium 1 hr after infecting cells with vaccinia virus. Here and in Tables 2 and 3, + denotes presence of virus and - denotes absence of vi s.

Table 2

Synthesis of vaccinia virus following introduction of histone at various intervals after infection

Time of introduction of histone	Titer of virus				
Simultaneously with virus					
After 30 min.					
" 1 hr.					
" 2 hr.					
" 4 hr.					
" 6 hr.					
" 8 hr.					
" 18 hr.					
Control (without histone)					

Note: Infective dose of vaccinia virus was 12 ID per cell.

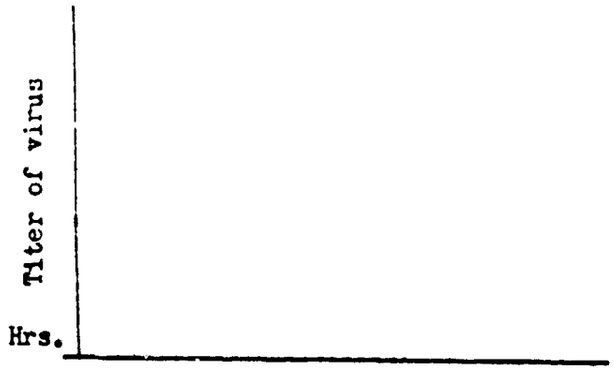
Table 3

Synthesis of vaccinia virus in cells pretreated with histone

Time elapsed from introduction of histone till infection (in hours)	Titer of virus			

Note: Infective dose of vaccinia virus was 0.1 ID per cell.

(Fig. of p. 24)



Trend of multiplication and accumulation of virus.

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(Received 8 May 1965)